

The University of Chicago

RAT SEMINAL VESICLES AND PROS-
TATE GLANDS AS QUANTITA-
TIVE INDICATORS OF TES-
TICULAR HORMONE

A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE BIO-
LOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF ZOOLOGY

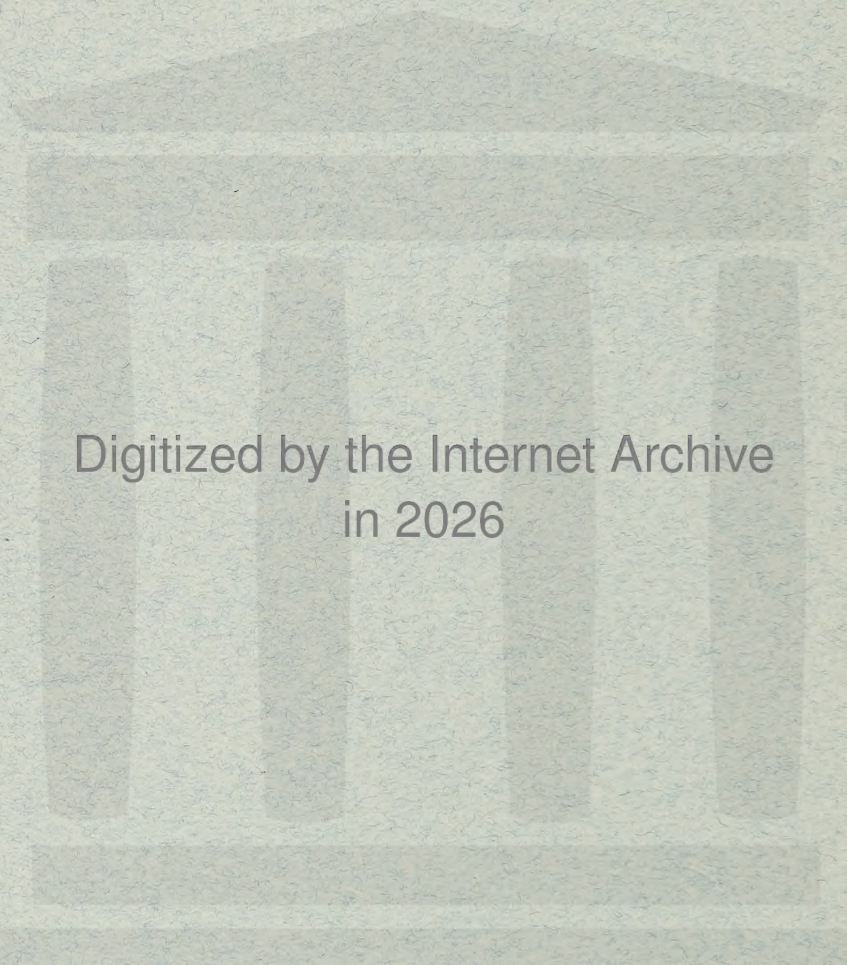
1932

By

IRA BOWERS HANSEN

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RAT SEMINAL VESICLES AND PROSTATE GLANDS AS QUANTITATIVE INDICATORS OF TESTICULAR HORMONE*

IRA BOWERS HANSEN

From Hull Zoological Laboratory, University of Chicago
CHICAGO

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I. INTRODUCTION

A study of the reactions of both seminal vesicles and prostate glands of rats was undertaken to determine their usefulness as quantitative indices of the presence of testicular hormone. It was known that both glands responded distinctly to castration with a definite histology that differed widely from the normal condition in those organs. It became desirable to know whether either or both glands would respond quantitatively to different concentrations of injected male hormone, and whether they would respond constantly enough so that they might be relied upon to be used as an assay method.

In the extraction of testicular hormone, active material is obtained, in a form as pure as possible, by successive treatments that eliminate inactive organic substances. The strength of activity, as well as the determination of the presence of activity in a solution, thus far can be indicated only by biologic tests. In the absence of chemical or physical tests for the estimation of the amount of active male hormone present in a sample, it is necessary to develop biologic tests that by their accuracy may be of distinct service to the physiological chemist or to the experimental worker who desires to know the strength of the male hormone he wishes to use. Thus far the chief criterion for such a purpose has been the capon comb, a quantitative method developed by Gallagher and Koch (1930).

The capon comb method is one that depends upon a reaction in the bird to substances extracted from mammalian tissues, and is thus far the most satisfactory method developed for assay. Suitable quantitative methods using the mammal as the test animal, however, are also desirable from several points of view. The comb growth reaction, though the most de-

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pendable assay method yet developed, is surrounded by certain features of a somewhat disconcerting nature. Further, radically different biological reactions may be important in determining the character of materials subjected to analysis. Thus both extracts of bull testes and extracts of male urine stimulate growth of the capon comb. Probably the same substance is obtained from both sources, but due to a slight uncertainty that it is so, analysis by bird and mammal reactivity would be desirable. It is also true that the maintenance of a proper group of capons for assay purposes is attended, perhaps, with greater difficulty and expense, at least in some localities, than is that of small laboratory animals. With these points in mind a study has been made of certain accessory reproductive organs in the rat to determine their applicability to the quantitative assay of the testis hormone.

In order to develop a quantitative method, it was necessary to find some tissue or organ that depends upon the testicular hormone for its normal activity and structure, and at the same time responds very definitely to castration. This requirement was easily filled by the rat seminal vesicles and prostate glands, the reactions of which to castration and to hormone injections had previously been extensively studied and reported by Moore, Hughes and Gallagher (1930) for the seminal vesicles, and by Moore, Price and Gallagher (1930) for the prostate.

To review briefly the situation as previously described, it can be said that the normal paired seminal vesicles of the rat are lobulated, elongated, sac-like glands that have a main central chamber running the entire length of the gland, from which diverge numerous side branches. The glandular epithelium is one cell in thickness, and the cells are of a tall columnar variety. The most conspicuous feature of the cytoplasm of these cells is the presence of secretory granules, usually numerous, between the basal nucleus and the distal end of the cell. These granules are characterized by the presence of encircling halos.

Following castration, Moore, Hughes and Gallagher (1930) point out that the enhanced secretory granules disappear within forty-eight hours, and that a progressive diminution in the height of the epithelium takes place until the cells are of the dimensions of the nucleus. Macroscopically there is likewise a decrease in the total size of the seminal vesicles, and a loss of secretory ability. These regressive involutionary changes have been stopped and a completely castrate gland rebuilt to the normal structural and functional state by the injection subcutaneously of extracts of bull testes. This definite reaction of the seminal vesicles to castration and their response to the presence of testicular hormone constitutes one of the major qualitative tests for male hormone.

In like manner, Moore, Price and Gallagher (1930) have described the normal prostate and its reactions to castration. The normal rat prostate consists of three lobes—anterior, middle, and posterior. The anterior portion is divided into a right and left lobule which is located on the posterior

margin of the respective left and right seminal vesicles. The anterior portion of the prostate is different histologically and physiologically from the middle and posterior lobes, which are identical. Since in the present study, the anterior prostate was omitted from consideration, it is pertinent only to indicate the normal and castrate conditions of the middle and posterior lobes.

The prostate is a typical acinous gland consisting of a number of convoluted alveoli supported by a stroma of loose connective tissue. In the normal condition the lining epithelium consists of a single layer of high columnar cells with basal nuclei. These cells are characterized by the presence of a light area in the distal portion of the cytoplasm between the nucleus and the outer cell wall. These light areas in the cells of the epithelium give the appearance under low magnification of a light band between the deeply stained nuclei and the cell border.

After castration it was pointed out that the light areas disappeared from the cells within five days, and that there was a definite loss in epithelial height. Besides these changes, there was a decrease in the size of the acini, and a decided increase in the amount of interacinous connective tissue. All these castration effects could be eliminated or prevented by the injection of extract of bull testicles. These reactions constitute a second qualitative test for the presence of male hormone.

With such adequate information available, it was quite obvious that the two glands gave a range of characteristics, not only in the number of different things responding to the lack of male hormone, but in the degree to which they reacted. To make use of them in a quantitative study it remained to discover what was the basal level following castration, how soon that basic condition developed following the removal of the gonads, whether it was more advantageous to inject immediately after the operation of gonadectomy, and lastly, what the quantitative responses might be in the tissues studied.

II. MATERIAL AND METHODS

To determine how soon the castration changes had become maximum and uniform, rats were killed at periods ranging from five days after castration, through ten, fifteen, twenty and sixty days following castration. It was found that seminal vesicles and prostates of five-day castrate rats had not completed the involutionary changes following the removal of the testes. It was likewise true that after ten days the histologic picture was not that of the basic level obtainable by longer periods of castration. At the end of fifteen days the histologic picture was decidedly castrate in the majority of animals. Complete uniformity was obtained after twenty days. Beyond that point there was a very small further change which was of no material importance. A sixty-day castrate does not differ noticeably from a twenty-day castrate, although a one hundred twenty-day castrate does show further decrease in size of cells beyond that shown in the twenty-day

castrate. The greatest involution, however, occurs safely within the first twenty days, and the stage reached at the end of that time may be used as a basic castrate condition upon which to rebuild the epithelium by hormone injections if so desired.

It was next necessary to discover whether it was advisable to start the injections immediately after castration, or following the assumption of the basic castrate condition, or at some time in between. The possibility existed that the length of time intervening between the gonadectomy and the beginning of injections would have a bearing upon the rapidity and the amount of response in both the seminal vesicles and the prostates. Likewise the question as to the number of daily injections to be given in order to get the most uniform picture among the animals had to be answered. Five animals were arbitrarily chosen as the minimum number to be used for the assay of any particular sample, and experience has shown that not fewer than five can be used without the sacrifice of some accuracy.

A preliminary group of animals was castrated and divided into four categories. Those in group A were permitted to remain ninety days before injections were started; group B for twenty days; group C for ten days; and a group D for only one day. Each group in turn was divided into two classes, one receiving ten consecutive injections, and the other receiving twenty consecutive daily injections. Each animal received one-half cc. injection daily of seven to eight bird units. This study indicated that the longer the time elapsing after castration, the longer time did it take to rebuild the gland. This was well shown in a comparison of the ten-day castrates with the ninety-day castrates. Likewise, there was good evidence to show that there was greater variation between the histologic pictures of the glands after twenty injections than after ten, a fact which probably can be explained upon the basis of poorer absorption as the amount of oil under the skin increases with added injections.

As a direct outcome of this preliminary study it was decided that ten daily injections beginning the day after castration, the so-called "maintenance procedure," gave less variable results, and was advantageous in that it reduced the time requirement for the total procedure, required less total hormone, and did not demand a stock supply of castrated rats.

The method that has been followed throughout this whole study, therefore, is as follows. Rats castrated were injected once daily beginning the day following the operation, for ten days with one-half cc. of the hormone sample. Upon the eleventh day they were killed and the seminal vesicles and prostates recovered. Before removal from the animal, the seminal vesicles were measured carefully with calipers and their condition noted as to turgidity and the presence of secretion. Fixation was in Bouin's solution. The tissue was cut at four miera and stained in Ehrlich's hematoxylin without counterstain.

Subsequent to the decision on a method of procedure, twenty-two samples of male hormone derived from bull testicular extracts and carried in

olive oil have been injected into batches of five animals each. Eighteen of these samples were dilutions of the same hormone stock. In addition two blanks consisting of pure olive oil have been injected, which constitutes in total the utilization of one hundred twenty rats. There has been no attempt to use inbred strains other than exists in a mixed colony maintained for purposes of reproduction. Rats that were used varied in weight from 170 to 380 grams.

III. QUANTITATIVE REACTIONS OF THE SEMINAL VESICLES

Macroscopically, the seminal vesicle responds to castration by becoming very flabby, decreasing in size, and by losing its power to secrete. This is in marked contrast with the normal gland which is usually turgid, distended, and full of coaguable secretion. Such obvious changes are qualitative criteria for the presence or absence of testicular hormone, but practically useless as a means of quantitative estimation of the amount of hormone the animal is receiving in its daily injections. There is a tremendous variation in the size of seminal vesicles in normal rats, so much so that size alone is of no use to indicate hormone concentrations. The size of the seminal vesicles has been carefully followed in an attempt to correlate the length-breadth index with the hormone strength, but the results are discouraging. Table I indicates the variation encountered in this method for the rat. The positive correlation between the bird units injected and the length-breadth index is 0.51.

TABLE I

Bird Units Injected	Length x Breadth of Left Seminal Vesicle
0.09	81.9
0.29	75.0
0.50	69.2
0.58	75.6
0.96	85.1
1.10	82.7
1.41	80.7
1.55	67.3
1.65	103.6
2.00	91.8
2.25	92.2
2.25	70.5
2.50	83.5
3.75	102.3
3.75	87.1
4.14	91.5
5.00	83.9
5.50	97.2
10-day castrate.....	70.9

Presence or absence of secretion, however, may serve as a general guide at the time of killing. Gentle pressure upon the gland after cutting from the animal may or may not result in the exudation of a secretion from the open lumen. If secretion does appear it may be watery or it may be viscous. Viscous secretion has been noted to be present in seminal vesicles after the injection of 1.65 bird units in all of the animals. Below that concentration secretion may appear in a single animal of a group but it is

always very watery and very scant in quantity. Microscopic examination shows some type of secretion to be present in the lumina of all vesicles, whether from castrates or from heavy injections, although varying in amount and in quality. That in a castrate is very fibrous, thin looking, and pale staining, and probably is a deterioration product of the secretion present before castration. In contrast, that from an animal receiving injections of 2.25 bird units shows a solid colloid more heavily staining with hematoxylin, a product of continued secretory activity. Microscopic examination for colloid is useless for a quantitative estimation of the units of hormone injected, while macroscopic examination seems to be good to indicate either a relatively high concentration, or a relatively low one.

In a consideration of the reaction of the seminal vesicle to varying concentrations of testicular hormone, there are certain cytologic characteristics which respond quantitatively and have been used to distinguish one dilution of hormone from another. The most obvious of these is the epithelial height. The epithelium of the normal rat seminal vesicle is of the tall columnar variety with an average height of eighteen to twenty micra. The cells are very narrow, about the width of the basally situated nucleus. Castrated animals, however, show an epithelium which in height is scarcely more than that of the nucleus. There is a range, then, from the castrate type (about 8.4 micra in the ten-day castrate) to the normal type of twenty micra.

For every sample assayed the average epithelial height has been determined by measuring with an adjustable ocular micrometer sixty cells in a random section for each animal. Five animals, then, permit a point based upon three hundred measurements, which is as accurate as the variability of the tissue will allow. Care was exercised to measure regions of the gland that were not distended by large amounts of secretion, and cells that were cut as nearly as possible in a plane parallel with the long axis of the particular cell. Table II and the graph of the seminal vesicle show clearly the results in summary form. There is a positive correlation between the

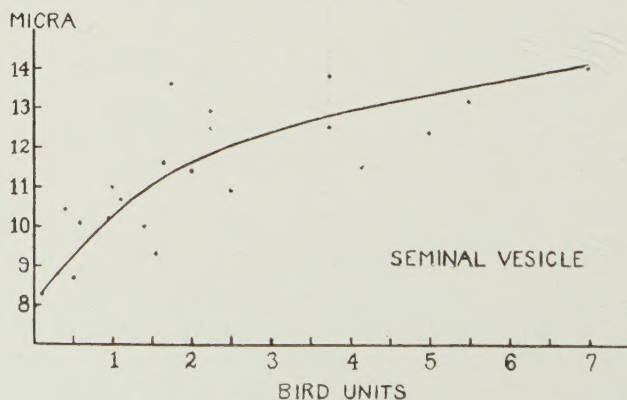


Figure 1. Correlation between Epithelial Height in Micra and the Strength of Hormone Injected in Bird Units for the Seminal Vesicle.

TABLE II
SEMINAL VESICLES

Bird Units Injected	Epithelial Height in Micra
0.09	8.3
0.29	8.8
0.40	10.4
0.50	8.7
0.58	10.1
0.96	10.2
1.00	11.0
1.10	10.7
1.41	10.0
1.55	9.3
1.65	11.6
1.75	13.6
2.00	11.4
2.25	12.9
2.25	12.5
2.50	10.9
3.75	12.5
3.75	13.8
4.14	11.5
5.00	12.4
5.50	13.2
7.00	14.0
10-day castrate.....	8.4
178-day castrate.....	7.2
Normal rat.....	20.0

strength of hormone injected and the epithelial height of 0.72 with a probable error of 0.069.

Another criterion that is of service in the estimation of the weakness or strength of hormone is the presence or absence of secretory granules. The cells of the normal seminal vesicle epithelium contain distinct secretory granules in the distal portion of the cells, and each of these granules is surrounded by a characteristic clear ring or halo. Castrated animals, however, have lost these granules possessing halos, and in this regard again present a distinctly different cytologic picture from the normal. Granules without halos have been shown to be present in seminal vesicles after receiving ten injections of a sample of 0.5 bird unit. These fine granules are difficult to see, and are of doubtful value to the assay. Granules showing halos are of service since they are differentiated from the ordinary granular cytoplasm, and it is these that are looked for in the determination of the strength of the sample. These were first noted in a small percentage of cells after an injection of 1.1 bird units. Granules increase in number and in strength as the concentration of the injected hormone is increased. This may be indicated roughly as shown in Table III.

TABLE III

Bird Units	Granules
1.10	— Small in size, few with tiny halos
1.41	— Weak, with tiny halos
1.55	— Weak, with few tiny halos
1.65	— Weak, small halos
2.00	— Weak
2.25	— Good, numerous
2.50	— Good, numerous
3.75	— Very distinct and numerous
4.14	— Very distinct and numerous
5.00	— Strikingly clear
5.50	— Strikingly clear

Granules are difficult to estimate quantitatively with any accuracy, but may be used to place an unknown sample in the general range of concentration.

Taking all the criteria into consideration, it is found that the seminal vesicle may be used as a quantitative index of testicular extract injections. It is available for assay of dilutions from about 0.4 bird unit up to approximately 7.0 bird units. The more accurate range of the seminal vesicles is considerably narrower and lies between 0.4 and 2.5 bird units. At higher concentrations the assay becomes less dependable.

IV. QUANTITATIVE REACTIONS OF THE PROSTATE GLAND

In this consideration it is to be borne in mind that the anterior lobe of the prostate (the "coagulating gland") does not enter into the discussion. Only the middle and posterior lobes have been followed, and the description belongs to their behavior. They have been treated as identities which seems strictly to be the case.

Macroscopically, the prostate in the normal rat is turgid, large, and full of secretion. Upon castration, changes occur which result in a smaller, darker colored gland without secretion. Usually there develops an abundance of fat upon and around the surface of the castrate prostate, a feature which is missing in the normal rat. Such changes are useful as a very general indication of the state of the hormone condition, but no more than that.

The prostate has a very characteristic cytology which is of use in an assay. The normal cells are tall columnar and are striking because of the presence of circumscribed light areas, or light spots, in the distal end of the cells. These areas stand out distinctly in contrast to the remainder of the granular cytoplasm. After castration these light areas totally disappear. There is, likewise, a concurrent involution of the epithelium resulting in a low cuboidal cell layer. Cells which normally average thirty-one micra drop in height to sixteen micra. With these castration changes there is a positive increase in the amount of connective tissue stroma between the acini of the gland. In regard to the stroma, the connective tissue response has not proved to be capable of more than a suggestive indication of strength. It is obvious, however, that it is only when the injected dose is well below one bird unit that the connective tissue stroma increases, and then not markedly until almost no hormone is administered. A very little testicular hormone is able to prevent the increase of connective tissue, although it may not keep the whole gland functional.

The prostate shows a reaction to low dosages of hormone that is rather difficult to record. Invariably it is found that the acini along the periphery of the glandular mass will respond first, and give a strong reaction while the rest of the gland remains castrate in all appearance with the exception of the amount of connective tissue. This response, then, is not uniform throughout the whole gland, but in weak concentrations shows itself in

only a relatively few acini. Likewise, in animals receiving relatively strong doses, the same difficulty prevails, due, however, to a different cause. Here the factor of distention plays a large rôle. An acinus that is distended with its secretory product will be found to be bordered by a lining epithelium of a cuboidal nature, in some cases still showing light areas, and in other cases without light areas and precisely similar to the cells found in an untreated total castrate. To take an average height of the epithelium as was done for the seminal vesicle is practically of no avail. To make an estimation of the percentage of cells, or acini, showing a definite reaction to the amount of non-reacting tissue can be done, but it is found that such a ratio in one animal will vary widely from the ratio in the second, a result which is most unsatisfactory where both animals are receiving the same concentration of testicular hormone. Further, the percentage of reacting tissue seems to bear little correlation with the hormone dosage.

To make some sort of estimation it has been found useful to measure the highest rather than an average epithelium. This method gives an indication of the strength of reaction in those portions of the gland which are actively responding. Ten measurements per gland have been taken of the highest epithelium available, and these measurements in turn averaged for each batch of five animals. The results are shown in Table IV and pictured in the graph. The positive correlation between the height of the cells as measured, and the strength of hormone injected, is 0.78 with a probable error of 0.056.

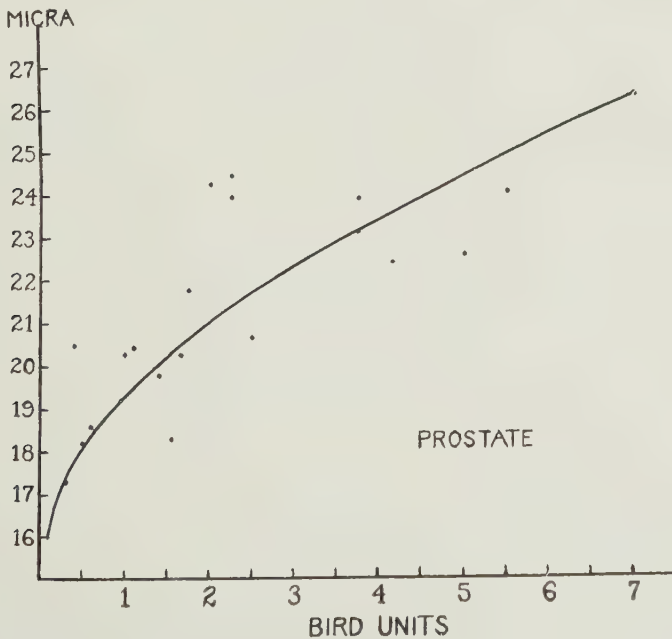


Figure 2. Correlation between Epithelial Height in Micra and the Strength of Hormone Injected in Bird Units for the Prostate Gland.

TABLE IV
PROSTATE GLAND

Bird Units Injected	Epithelial Height in Micra
0.09	16.0
0.29	17.3
0.40	22.6
0.50	18.2
0.58	18.6
0.96	19.2
1.00	23.3
1.10	22.3
1.41	19.8
1.55	18.3
1.65	20.3
1.75	21.8
2.00	24.3
2.25	24.5
2.25	24.0
2.50	20.7
3.75	24.0
3.75	23.2
4.14	22.5
5.00	22.7
5.50	24.2
7.00	26.5
10-day castrate.....	15.9
Normal rat.....	31.0

Tissue which shows no light areas at all must be relegated to the castrate category, not because it is impossible to distinguish it in many cases from a typical twenty-day castrate, but because it is impossible to separate it from a ten-day castrate, the control period for these experiments. It was found that a ten-day castrate prostate shows variations in epithelium height sufficient to make it impossible to decide between a very low concentration of hormone (0.09 bird unit) and the castrate type itself. The weakest dose that has thus far been observed to produce light areas is 0.29 bird unit, but it is believed that they will appear at lower concentrations than that.

In general, as the hormone strength increases there is a progressive increment in the number of acini reacting until even the distended acini will show cuboidal epithelium containing distinct light areas. These light areas first appear weak, more easily seen under lower magnifications than under oil immersion, and then increase in contrast, in sharpness, and in size until they are strikingly obvious even under oil. The assay range of the prostate is rather short, for beyond 1.5 bird units the response begins to approach a maximum that does not permit clear distinctions; for example, between a hormone strength of two bird units and one of four bird units.

V. APPLICABILITY OF THE METHOD

After the method of procedure had been established, twenty-two hormone samples plus two blanks of pure olive oil were injected into rats as unknowns. Although previously carefully assayed in bird units by Dr. T. F. Gallagher, the values were kept secret until a report was made both placing the unknowns in their relative order of strength, and assigning a value to them upon the basis of the reactions of both seminal vesicles and

prostate glands. This prevented any prejudice or any preconceived notion of what the tissue should be like from entering into the actual judgment and calculation. It also indicated roughly how useful and how dependable for an assay were such criteria as have been described. The first series of five unknown samples were merely placed into order according to the relative strength of hormone as indicated by the tissue reactions of the seminal vesicles and prostates. This proved easy to do since the five samples were wide in concentration range, and in actual values were 1.1, 2, 3.75, 5.5 bird units and one blank of pure olive oil.

With these four points established, another series of five unknowns were injected and a report made with reference to their relative strengths and estimated concentrations in bird units. Since nothing lower than 1.1 bird units had previously been given, those doses reported in bird units below that were largely chance judgments with the exception that they were definitely weaker than 1.1 bird units. They were again placed in the correct order of strength. A comparison of actual value and estimated value may be made in the following table.

SERIES 2

Actual Value in Bird Units	Estimated Value in Bird Units
0.00	0.3
0.58	0.7
0.96	0.9
1.65	4.0
2.25	4.5

The procedure was repeated with five more unknowns, now using the additional data acquired as further reference points. Again the correct order was reported for the concentrations, and can be summed up in the following table. It will be noted that 2.25 bird units appears in both series 2 and series 3, and were portions of the same sample.

SERIES 3

Actual Value in Bird Units	Estimated Value in Bird Units
0.09	0.00
0.29	0.75
1.41	0.98
2.25	4.50
3.75	6.70

Series 4 is a repetition of the same procedure, using unknown samples, and with the use of previous experience, attempting to estimate the concentration in bird units. Again the samples were easily placed in order according to strength. This has proven to be true of all series thus far injected.

ASSAY OF TESTICULAR HORMONE

SERIES 4

Actual Value in Bird Units	Estimated Value in Bird Units
0.50	0.25
1.55	0.60
2.50	1.70
4.14	3.00
5.00	4.00

With the last group of animals into which four unknowns were injected, it was anticipated that a good estimation might be obtained of the actual adaptability of the seminal vesicles and prostate glands to use for an assay. Unfortunately the whole group of rats, during the course of the injections, became very sick with dysentery, and the results, therefore, are affected by the factor of ill health and do not represent the full potentialities of this method as an assay. The estimation and actual values are given in the following table.

SERIES 5

Actual Value in Bird Units	Estimated Value in Bird Units
0.40	1.4
1.00	1.8
1.75	5.7
7.00	6.5

VI. DISCUSSION

As a quantitative method, the several criteria of the seminal vesicles and prostate glands, taken separately or considered in the aggregate, are adaptable to use for an assay. Just as is the case with a large number of biologic assays depending upon the numerous uncontrollable factors running rampant in the living organism, the desired precision is still lacking and it is somewhat doubtful whether it will ever be attained much beyond its present status. It is assured that both the prostate and seminal vesicles react not by any "all or none" response to a requisite threshold, but in direct proportion to the amount of stimulating hormone present. Such a sensitivity to the presence of the existing agent is the fundamental property that permits of an assay based upon those tissues. It was shown by Moore and Gallagher (1930) that there was such a relationship existing for the prostate and seminal vesicles, and further, that there was a corresponding relation in the response of the Cowper's gland and the vas deferens. They brought out the minimum hormone threshold relationships between the various accessories and indicated clearly that the most sensitive, the organ responding to the least amount of testicular hormone, was the prostate and that this was followed in turn by the seminal vesicle, Cowper's gland, and finally the vas deferens with the highest hormone threshold.

This present study is able to corroborate fully the threshold relations of the seminal vesicle and the prostate. It has been shown that the prostate will react to exceedingly minute amounts of testicular extract. A bird unit of bull testicular hormone is equivalent to approximately 0.01 mgm. of solid active material (Gallagher and Koch, 1929). Nevertheless, characteristic light areas have appeared in some of the cells with a dose as low as 0.29 bird unit (daily injection of 0.002 mgm. of gross solids), and it is probable that the light areas can be produced by smaller injections than this. They did not appear after the injection of 0.09 bird unit, although the requisite epithelium height was sufficient to permit their appearance. The seminal vesicle, upon the other hand, shows but little response until 0.4 bird unit has been employed, and then the reaction consists merely in an increase in the cytoplasm with the concurrent increase in height of the epithelium. This condition is not a functional one by any means, nor are any of the cells in the gland in a functional state. This is in contrast with the prostate where it is believed that light areas are indicators of physiological function. Slight functional ability as indicated by secretory granules with halos appears about 1.1 bird units.

A further contrast between the seminal vesicles and the prostate is strongly brought out by the amount of hormone necessary to produce a healthy, functional gland. Two bird units will do it easily for the prostate, while about six to seven are needed for the seminal vesicle. It should be pointed out that while such dosages do produce a functional gland with the cytologic picture of the normal, it does not reproduce the epithelial height of the average normal rat. With the concentrations of testicular hormone used (up to seven bird units), it has proved impossible to duplicate the height of the epithelium of either the normal prostate or the normal seminal vesicle. This seems a little hard to explain when it is not necessary to rebuild an epithelium within ten days, but merely to sustain it, unless we logically admit that the average normal rat is producing greater than seven bird units per day.

Heller (1932) injected various concentrations of testicular hormone into castrate rats in the attempt to find what direct relationship there might be between hormone concentration and reaction of Cowper's gland. His table of data and the accompanying graph indicate quite clearly that there is a positive correlation certainly, but the gland is too slow in reacting, and too variable to serve as an assay method. He apparently was successful with either six or seven bird units in restoring the minimum normal height of twenty-nine micra, but never succeeded in reaching the upper range of thirty-two micra.

Moore and Gallagher (1930), after carefully studying threshold relationships of the accessories to testicular hormone, defined the rat unit in terms of complete gonadal substitution. They stated, "A rat unit of male hormone is defined as that minimal daily amount required to maintain fifty

per cent of castrated animals in normal condition," and stipulated that the indices of the normal condition be the normal prostate and seminal vesicle. At that time it was suggested that one rat unit was equal to approximately six bird units. Although this present study seems to indicate that six bird units is a bit too low an estimate for complete replacement of epithelial height, it nevertheless is not far in error.

The results of this study, obviously somewhat incomplete, do not especially recommend it in preference to the capon comb growth method. The latter method is easy to apply, and the assay is readily obtained by the simple measurement of the comb growth. Further, the same animals can be used again for subsequent assays as they are not sacrificed. The chief unfortunate features lie in the difficulty of performing a successful operation which will leave the capon permanently without testes, and in the care and space that chickens require in order not only to maintain a colony, but to keep the birds healthy. Upon the other hand, rats are available for use in nearly every laboratory, castration is rapid and sure, and the animals withstand surgical treatment without difficulty. There is the distinct disadvantage, however, that the experimental animals have to be sacrificed to make the assay, and that there must intervene the routine technique of fixing, sectioning, and staining, before the tissues can be studied. This is a handicap to speed, and although smaller amounts of hormone can be detected than by the use of the capon comb further work is necessary before one is justified in claiming great accuracy in assay. There may be further justification for the rat method in that a mammal is used as the test animal for a mammalian tissue product.

Other methods for indicating quantities of male hormone have been suggested. Funk, Harrow and Lejwa (1930) suggested a cock unit depending upon the reactions of capons. Their cock unit is defined as "that amount of male hormone which, when injected, will increase the size of the comb and wattles to the extent of 10 mm. in 10 days." Six animals are used for each test, and an average reaction taken. Schoeller and Gehrke (1931) also defined a bird unit depending upon the capon comb reaction, and Freud, de Fremery, and Laqueur (1932) have added still another bird unit depending upon the capon comb.

Muto (1928) suggested that the weight of the seminal vesicles in the mouse be used as an index for testicular hormone, since there is a decided difference between the normal condition and that following upon castration.

Martins and Rocha e Silva (1930) indicated that a more useful index of hormone concentration might be found in the utilization of measurements of the mouse seminal vesicle. They measured the length and the breadth, and derived a length-breadth index by multiplying the two together. In the normal eighteen-gram mouse, the index varies between fifty-two and ninety-two. Ten days after castration the index is as low as

twenty and sometimes as low as ten. After hormone injection they were able to build a seminal vesicle with an index of sixty. The range between twenty and sixty should serve for an assay method.

Loewe and Voss (1930) in a review article, outline their "cytologic regeneration" test for male hormone. Voss and Loewe (1931) give further information. They indicate that macroscopic relations of the mouse seminal vesicle are too variable for an assay method, but devote their energies to a microscopic determination. Following a previous castration period of four weeks, the mice are injected three times in thirty-six hours and killed one hundred hours after the first injection. For an assay they depend upon secretion granules and vacuoles, cell height, new mitotic figures, secretion in the lumen, and the condition of the chondriosomes. In the 1931 paper they publish an interesting graph plotting the correlation between the secretory granules and the length-breadth index. They roughly classify the granular condition into five categories, granules in all cells, in most cells, in one-half of the cells, in a few cells, and in no cells. This curve, then, can be but approximate and depend very largely upon personal judgment of the investigator. The cytologic regeneration test is an interesting one, and closely associates itself with the present study upon the rat. Just how precise the mouse test is, is not known to this author.

Lendle (1931) has attempted to make use of the anti-feminine reaction to establish a standard quantity of male hormone. The amount of male hormone necessary to suppress the oestrous cycle in female rats is the unit involved. Ihrke and D'Amour (1931) also showed clearly that injection of male hormone would suppress the oestrous cycle, but made no attempt to use such information as a measure of hormone strength. There appears to be one difficulty at least in the way of a safe assay thereby, and that lies in the fact that many factors will affect oestrous, and particularly the injection of organic impurities.

Glaser and Haempel (1931), working with fish, announced a method to standardize male hormone by injecting it into a fish, *Rhodeus amarus*, which had been castrated thirty days or more in advance. Within four to five hours after injection there is an intense red coloration of the fish. This method has the advantage of a five-hour standardization, but again probably is not very precise. This far only a note has been published concerning the reaction.

Even though male hormone has been produced in crystalline form by Butenandt (1932), there still is interest in a quantitative assay method. For the mammals thus far, only the method of Loewe and Voss, and the present study seem to give promise. Both are similar, with the exception that by the use of both seminal vesicles and prostate glands a wider range for assay is obtained, smaller dosages of hormone can be determined, and the two glands serve as a check upon each other.

VII. SUMMARY AND CONCLUSIONS

In a study of the applicability of rat seminal vesicles and prostate glands to the assay of testicular hormone, it was found that both structures gave quantitative reactions that might be used as criteria of hormone strength. The indices of the seminal vesicle were the presence of secretion, cell height, and the presence of secretory granules with halos. For the prostate, epithelial height and the presence of light areas proved satisfactory. In a comparison of the prostate and seminal vesicle it was shown that the prostate was the more sensitive with an accurate assay range from about 0.15 bird unit up to 1.5 bird units. The seminal vesicle indicated an assay range varying from 0.4 bird unit up to approximately 7.0 bird units. By using both glands in combination the assay range extended from 0.15 bird unit to 7.0 bird units. These conclusions are based upon the assay of 24 samples that had been previously assayed in bird units.

A method of procedure was developed for the assay of testicular hormone. This consisted in total castration of five rats upon one day and injection subcutaneously of one-half cc. daily of the unknown sample, beginning the day after operation, for ten successive injections. Upon the eleventh day the animals were killed and the seminal vesicles and prostates recovered. Tissue was fixed in Bouin's, cut at four micra and stained in Ehrlich's hematoxylin without counterstain.

This problem was suggested by Dr. Carl R. Moore, and it is with much gratitude that his advice, guidance and assistance are acknowledged. The extracts of bull testicles employed in this study were prepared by Dr. T. F. Gallagher under the direction of Professor F. C. Koch in the Department of Physiological Chemistry and Pharmacology, and had been previously assayed by Dr. Gallagher in bird units. It is indeed a great pleasure to acknowledge to them my indebtedness for the material and cooperation in this work.

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